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(57) Abstract

The subject invention pertains to novel insecticidal toxins and genes which encode these toxins. Also disclosed are novel nucleotide primers for the identification of genes encoding toxins active against pests. The primers are useful in PCR techniques to produce gene fragments which are characteristic of genes encoding these toxins.

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DESCRIPTION

BACILLUS THURINGIENSIS TOXINS

Background of the Invention

The soil microbe *Bacillus thuringiensis* (*B.t.*) is a Gram-positive, spore-forming bacterium traditionally characterized by parasporal crystalline protein inclusions. These inclusions often appear microscopically as distinctively shaped crystals. The proteins can be highly toxic to pests and specific in their toxic activity. Certain *B.t.* toxin genes have been isolated and sequenced, and recombinant DNA-based *B.t.* products have been produced and approved for use. In addition, with the use of genetic engineering techniques, new approaches for delivering *B.t.* toxins to agricultural environments are under development, including the use of plants genetically engineered with endotoxin genes for insect resistance and the use of stabilized intact microbial cells as *B.t.* toxin delivery vehicles (Gaertner, F.H., L. Kim [1988] *TIBTECH* 6:S4-S7; Beegle, C.C., T. Yamamoto, "History of *Bacillus thuringiensis* Berliner research and development," *Can. Ent.* 124:587-616). Thus, isolated *B.t.* toxin genes are becoming commercially valuable.

Until the last fifteen years, commercial use of *B.t.* pesticides has been largely restricted to a narrow range of lepidopteran (caterpillar) pests. Preparations of the spores and crystals of *B. thuringiensis* subsp. *kurstaki* have been used for many years as commercial insecticides for lepidopteran pests. For example, *B. thuringiensis* var. *kurstaki* HD-1 produces a crystalline δ-endotoxin which is toxic to the larvae of a number of lepidopteran insects.

Investigators have now discovered *B.t.* pesticides with specificities for a much broader range of pests. For example, other species of *B.t.*, namely *israelensis* and *morrisoni* (a.k.a. *tenebrionis*, a.k.a. *B.t.* M-7, a.k.a. *B.t. san diego*), have been used commercially to control insects of the orders Diptera and Coleoptera, respectively (Gaertner, F.H. [1989] "Cellular Delivery Systems for Insecticidal Proteins: Living and Non-Living Microorganisms," in *Controlled Delivery of Crop Protection Agents*, R.M. Wilkins, ed., Taylor and Francis, New York and London, 1990, pp. 245-255.). See also Couch, T.L. (1980) "Mosquito Pathogenicity of Bacillus thuringiensis var. israelensis," *Developments in Industrial Microbiology* 22:61-76; and Beegle, C.C. (1978) "Use of Entomogenous Bacteria in Agroecosystems," *Developments in Industrial Microbiology* 20:97-104. Krieg, A., A.M. Huger, G.A. Langenbruch, W. Schnetter (1983) *Z. ang. Ent.* 96:500-508 describe *Bacillus thuringiensis* var. *tenebrionis*, which is reportedly active

against two beetles in the order Coleoptera. These are the Colorado potato beetle, *Leptinotarsa decemlineata*, and *Agelastica alni*.

More recently, new subspecies of *B.t.* have been identified, and genes responsible for active δ-endotoxin proteins have been isolated (Höfte, H., H.R. Whiteley [1989] *Microbiological Reviews* 52(2):242-255). Höfte and Whiteley classified *B.t.* crystal protein genes into four major classes. The classes were CryI (Lepidoptera-specific), CryII (Lepidoptera- and Diptera-specific), CryIII (Coleoptera-specific), and CryIV (Diptera-specific). The discovery of strains specifically toxic to other pests has been reported (Feitelson, J.S., J. Payne, L. Kim [1992] *Bio/Technology* 10:271-275). CryV has been proposed to designate a class of toxin genes that are nematode-specific. Lambert *et al.* (Lambert, B., L. Buysse, C. Decock, S. Jansens, C. Piens, B. Saey, J. Seurinck, K. van Audenhove, J. Van Rie, A. Van Vliet, M. Peferoen [1996] *Appl. Environ. Microbiol* 62(1):80-86) describe the characterization of a Cry9 toxin active against lepidopterans. Published PCT applications WO 94/05771 and WO 94/24264 also describe *B.t.* isolates active against lepidopteran pests. Gleave *et al.* ([1991] *JGM* 138:55-62), Shevelev *et al.* ([1993] *FEBS Lett.* 336:79-82; and Smulevitch *et al.* ([1991] *FEBS Lett.* 293:25-26) also describe *B.t.* toxins. Many other classes of *B.t.* genes have now been identified.

The cloning and expression of a *B.t.* crystal protein gene in *Escherichia coli* has been described in the published literature (Schnepf, H.E., H.R. Whiteley [1981] *Proc. Natl. Acad. Sci. USA* 78:2893-2897.). U.S. Patent 4,448,885 and U.S. Patent 4,467,036 both disclose the expression of *B.t.* crystal protein in *E. coli*. U.S. Patents 4,990,332; 5,039,523; 5,126,133; 5,164,180; and 5,169,629 are among those which disclose *B.t.* toxins having activity against lepidopterans. PCT application WO96/05314 discloses PS86W1, PS86V1, and other *B.t.* isolates active against lepidopteran pests. The PCT patent applications published as WO94/24264 and WO94/05771 describe *B.t.* isolates and toxins active against lepidopteran pests. *B.t.* proteins with activity against members of the family Noctuidae are described by Lambert *et al., supra*. U.S. Patents 4,797,276 and 4,853,331 disclose *B. thuringiensis* strain *tenebrionis* which can be used to control coleopteran pests in various environments. U.S. Patent No. 4,918,006 discloses *B.t.* toxins having activity against dipterans. U.S. Patent No. 5,151,363 and U.S. Patent No. 4,948,734 disclose certain isolates of *B.t.* which have activity against nematodes. Other U.S. patents which disclose activity against nematodes include 5,093,120; 5,236,843; 5,262,399; 5,270,448; 5,281,530; 5,322,932; 5,350,577; 5,426,049; and 5,439,881.

A *cry*2Aa gene from HD263 *kurstaki* is disclosed by Donovan *et al.* in 264 JBC 4740 (1989). Another *cry*2Aa gene and a *cry*2Ab gene, from HD1 *kurstaki*, are disclosed by Widner & Whiteley, 171 *J. Bac.* 965-974 (1989). Another *cry*2Ab gene from HD1 *kurstaki* is disclosed

by Dankocsik *et al.* in 4 *Mol. Micro* 2087-2094 (1990). A *cry*2Ac gene from *B.t.*S-1 (*shanghai*) is disclosed by Wu *et al.* in 81 FEMS 31-36 (1991).

An isolate known as PS192M4 is disclosed in U.S. Patent No. 5,273,746 as having activity against lice.

The PS86I2 isolate is disclosed in U.S. Patent No. 5,686,069 as having activity against lepidopterans. PS91C2 is exemplified therein as producing a *Cry*IF(b)-type of lepidopteran-active toxin, the sequence of which is disclosed therein.

Sequence information for a lepidopteran-active toxin from HD525 and the sequence of a lepidopteran-active toxin from HD573 are disclosed in WO 98/00546. Those toxins are not *Cry*2-type toxins.

As a result of extensive research and investment of resources, other patents have issued for new *B.t.* isolates and new uses of *B.t.* isolates. *See* Feitelson *et al.*, *supra*, for a review. However, the discovery of new *B.t.* isolates and new uses of known *B.t.* isolates remains an empirical, unpredictable art. U.S. Patent No. 5,506,099 describes methods for identifying unknown *B.t.* isolates. U.S. Patent No. 5,204,237 describes specific and universal probes for the isolation of *B.t.* toxin genes. These patents, however, do not describe the probes and primers of the subject invention.

Brief Summary of the Invention

The subject invention concerns materials and methods useful in the control of non-mammalian pests and, particularly, plant pests. In a specific embodiment, the subject invention provides new toxins useful for the control of lepidopterans. A preferred embodiment of the subject invention further provides nucleotide sequences which encode the novel lepidopteran-active toxins of the subject invention.

The subject invention further provides nucleotide sequences and methods useful in the identification and characterization of novel genes which encode pesticidal toxins. In one embodiment, the subject invention concerns unique nucleotide sequences which are useful as primers in PCR techniques. The primers produce characteristic gene fragments which can be used in the identification and isolation of novel toxin genes. A further aspect of the subject invention is the use of the disclosed nucleotide sequences as probes to detect genes encoding *B.t.* toxins which are active against lepidopterans.

Further aspects of the subject invention include other novel genes and toxins identified using the methods and nucleotide sequences disclosed herein, in addition to the novel genes and toxins specifically disclosed herein. The genes thus identified encode toxins active against

lepidopterans. Similarly, the isolates capable of producing these toxins have activity against these pests. Thus, the subject invention further provides new *Bacillus thuringiensis* isolates having pesticidal activities which are found with the primers and probes according to the subject invention.

In one embodiment of the subject invention, *B.t.* isolates can be cultivated under conditions resulting in high multiplication of the microbe. After treating the microbe to provide single-stranded genomic nucleic acid, the DNA can be contacted with the primers of the invention and subjected to PCR amplification. Characteristic fragments of toxin-encoding genes are amplified by the procedure, thus identifying the presence of the toxin-encoding gene(s).

In a preferred embodiment, the subject invention concerns plants cells transformed with at least one polynucleotide sequence of the subject invention such that the transformed plant cells express pesticidal toxins in tissues consumed by the target pests. Such transformation of plants can be accomplished using techniques well known to those skilled in the art and would typically involve modification of the gene to optimize expression of the toxin in plants. In addition, the toxins of the subject invention may be chimeric toxins produced by combining portions of multiple toxins.

As an alternative to the transformation of plants, the *B.t.* isolates and toxins of the subject invention, or recombinant microbes expressing the toxins described herein, can be used to control pests. In this regard, the invention includes the treatment of substantially intact *B.t.* cells, and/or recombinant cells containing the expressed toxins of the invention, treated to prolong the pesticidal activity when the substantially intact cells are applied to the environment of a target pest. The treated cell acts as a protective coating for the pesticidal toxin. The toxin becomes active upon ingestion by a target insect.

Brief Description of the Sequences

SEQ ID NO. 1 is a forward primer useful according to the subject invention.

SEQ ID NO. 2 is a reverse primer useful according to the subject invention.

SEQ ID NO. 3 is a nucleotide sequence which encodes the 192M4 toxin.

SEQ ID NO. 4 is the predicted amino acid sequence of the 192M4 toxin.

SEQ ID NO. 5 is a nucleotide sequence which encodes the HD573 toxin.

SEQ ID NO. 6 is the predicted amino acid sequence of the HD573 toxin.

SEQ ID NO. 7 is a nucleotide sequence which encodes the HD525 toxin.

SEQ ID NO. 8 is the predicted amino acid sequence of the HD525 toxin.

SEQ ID NO. 9 is a nucleotide sequence which encodes the 86I2 toxin.

SEQ ID NO. 10 is the predicted amino acid sequence of the 86I2 toxin.

Detailed Disclosure of the Invention

The subject invention concerns materials and methods for the control of non-mammalian pests. In specific embodiments, the subject invention pertains to new *Bacillus thuringiensis* toxins, and genes encoding toxins, which have activity against lepidopterans. The subject invention concerns not only the polynucleotide sequences which encode these toxins, but also the use of these polynucleotide sequences to produce recombinant hosts which express the toxins. The subject invention further concerns novel nucleotide sequences that are useful as primers and probes for *Bacillus thuringiensis* (*B.t.*) genes that encode pesticidal toxins, especially lepidopteran-active toxins. The subject invention still further concerns novel methods for identifying and characterizing *B.t.* isolates, toxins, and genes with useful properties.

The new toxins and polynucleotide sequences provided here are defined according to several parameters. One critical characteristic of the toxins described herein is pesticidal activity. In a specific embodiment, these toxins have activity against lepidopteran pests. The toxins and genes of the subject invention can be further defined by their amino acid and nucleotide sequences. The sequences of the molecules can be defined in terms of homology or identity to certain exemplified sequences as well as in terms of the ability to hybridize with, or be amplified by, certain exemplified probes and primers. The toxins provided herein can also be identified based on their immunoreactivity with certain antibodies.

Methods have been developed for making useful chimeric toxins by combining portions of *B.t.* crystal proteins. The portions which are combined need not, themselves, be pesticidal so long as the combination of portions creates a chimeric protein which is pesticidal. This can be done using restriction enzymes, as described in, for example, European Patent 0 228 838; Ge, A.Z., N.L. Shivarova, D.H. Dean (1989) *Proc. Natl. Acad. Sci. USA* 86:4037-4041; Ge, A.Z., D. Rivers, R. Milne, D.H. Dean (1991) *J. Biol. Chem.* 266:17954-17958; Schnepf, H.E., K. Tomczak, J.P. Ortega, H.R. Whiteley (1990) *J. Biol. Chem.* 265:20923-20930; Honee, G., D. Convents, J. Van Rie, S. Jansens, M. Peferoen, B. Visser (1991) *Mol. Microbiol.* 5:2799-2806. Alternatively, recombination using cellular recombination mechanisms can be used to achieve similar results. See, for example, Caramori, T., A.M. Albertini, A. Galizzi (1991) *Gene* 98:37-44; Widner, W.R., H.R. Whiteley (1990) *J. Bacteriol.* 172:2826-2832; Bosch, D., B. Schipper, H. van der Kliej, R.A. de Maagd, W.J. Stickema (1994) *Biotechnology* 12:915-918. A number of other methods are known in the art by which such chimeric DNAs can be made. The subject

invention is meant to include chimeric proteins that utilize the novel sequences identified in the subject application.

With the teachings provided herein, one skilled in the art could readily produce and use the various toxins and polynucleotide sequences described herein.

B.t. isolates useful according to the subject invention have been deposited in the permanent collection of the Agricultural Research Service Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, USA. The culture repository numbers of the *B.t.* strains are as follows:

Table 1.

B.t. Isolate	Repository No.	Deposit Date
PS86I2	NRRL B-21957	March 12, 1998
PS192M4	NRRL B-18932	December 27, 1991

Table 2.

Source Isolate	*E. coli* Strain	Plasmid	Repository No.	Deposit Date
PS192M4	MR908	pMYC2586	NRRL B-21631	October 17, 1996
HD573	MR909	pMYC2587	NRRL B-21632	October 17, 1996
HD525	MR910	pMYC2588	NRRL B-21633	October 17, 1996

Cultures have been deposited under conditions that assure that access to the cultures is available during the pendency of this patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 CFR 1.14 and 35 U.S.C. 122. The deposits will be available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

Further, the subject culture deposits will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, *i.e.*, they will be stored with all the care necessary to keep them viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposit, and in any case, for a period of at least thirty (30) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the culture(s). The depositor

acknowledges the duty to replace the deposit(s) should the depository be unable to furnish a sample when requested, due to the condition of a deposit. All restrictions on the availability to the public of the subject culture deposits will be irrevocably removed upon the granting of a patent disclosing them.

The isolates HD525 and HD573 are available fromm the USDA-ARS NRRL Culture Collection, Peoria, Illinois.

Following is a table which provides characteristics of certain isolates useful according to the subject invention.

Table 3. Description of native *B.t.* strains

Strain	Inclusion Type	H-Serotype	SDS-PAGE protein profile
192M4	Amorphic	4a4b, sotto	130, 68
86I2	Bipyramidal	8	130, 30, 15
HD525	Bipyramidal with ORT	not motile	130
HD573	Bipyramidal	not motile	140, 130, 70

Genes and toxins. The genes and toxins useful according to the subject invention include not only the full length sequences but also fragments of these sequences, variants, mutants, and fusion proteins which retain the characteristic pesticidal activity of the novel toxins specifically exemplified herein. Chimeric genes and toxins, produced by combining portions from more than one *B.t.* toxin or gene, may also be utilized according to the teachings of the subject invention. As used herein, the terms "variants" or "variations" of genes refer to nucleotide sequences which encode the same toxins or which encode equivalent toxins having pesticidal activity. As used herein, the term "equivalent toxins" refers to toxins having the same or essentially the same biological activity against the target pests as the exemplified toxins.

It should be apparent to a person skilled in this art that genes encoding active toxins can be identified and obtained through several means. The specific genes exemplified herein may be obtained from the isolates deposited at a culture depository as described above. These genes, or portions or variants thereof, may also be constructed synthetically, for example, by use of a gene synthesizer. Variations of genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as *Bal*31 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which encode active fragments may be

obtained using a variety of restriction enzymes. Proteases may be used to directly obtain active fragments of these toxins.

Equivalent toxins and/or genes encoding these equivalent toxins can be derived from *B.t.* isolates and/or DNA libraries using the teachings provided herein. There are a number of methods for obtaining the pesticidal toxins of the instant invention. For example, antibodies to the pesticidal toxins disclosed and claimed herein can be used to identify and isolate other toxins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the toxins which are most constant and most distinct from other *B.t.* toxins.

Fragments and equivalents which retain the pesticidal activity of the exemplified toxins would be within the scope of the subject invention. Also, because of the redundancy of the genetic code, a variety of different DNA sequences can encode the amino acid sequences disclosed herein. It is well within the skill of a person trained in the art to create these alternative DNA sequences encoding the same, or essentially the same, toxins. These variant DNA sequences are within the scope of the subject invention. As used herein, reference to "essentially the same" sequence refers to sequences which have amino acid substitutions, deletions, additions, or insertions which do not materially affect pesticidal activity. Fragments retaining pesticidal activity are also included in this definition.

Certain toxins of the subject invention have been specifically exemplified herein. Since these toxins are merely exemplary of the toxins of the subject invention, it should be readily apparent that the subject invention also relates to variants or equivalents of novel genes and toxins having the same or similar pesticidal activity of the exemplified novel toxins. Equivalent toxins will have amino acid homology with a novel exemplified toxin. These equivalent genes and toxins will typically have greater than 60% identity with the sequences specifically exemplified herein; preferably, there will be more than 75% identity, more preferably greater than 80%, most preferably greater than 90%, and the identity can be greater than 95%. The amino acid homology will be highest in critical regions of the toxin which account for biological activity or are involved in the determination of three-dimensional configuration which ultimately is responsible for the biological activity. In this regard, conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Table 4 provides a listing of examples of amino acids belonging to each class.

Table 4.

Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not significantly detract from the biological activity of the toxin.

The toxins of the subject invention can also be characterized in terms of the shape and location of toxin inclusions, which are described above. Although novel crystal proteins are specifically exemplified herein, isolates for use according to the subject invention can be grown under conditions that facilitate the secretion of toxins. Thus, the supernatant from these cultures can be used to obtain toxins according to the subject invention. Thus, the subject invention is not limited to crystal proteins; useful soluble proteins are also contemplated.

As used herein, reference to "isolated" polynucleotides and/or "purified" toxins refers to these molecules when they are not associated with the other molecules with which they would be found in nature. Thus, reference to "isolated and purified" signifies the involvement of the "hand of man" as described herein. Chimeric toxins and genes also involve the "hand of man."

The use of oligonucleotide probes provides a method for identifying the toxins and genes of the subject invention, and additional novel genes and toxins. Probes provide a rapid method for identifying toxin-encoding genes. The nucleotide segments which are used as probes according to the invention can be synthesized using a DNA synthesizer and standard procedures, for example.

Recombinant hosts. The toxin-encoding genes of the subject invention can be introduced into a wide variety of microbial or plant hosts. Expression of the toxin gene results, directly or indirectly, in the production and maintenance of the pesticide. With suitable microbial hosts, *e.g.*, *Pseudomonas*, the microbes can be applied to the situs of the pest, where they will proliferate and be ingested. The result is a control of the pest. Alternatively, the microbe hosting the toxin gene can be killed and treated under conditions that prolong the activity of the toxin and stabilize the cell. The treated cell, which retains the toxic activity, then can be applied to the environment of the target pest.

A wide variety of methods are available for introducing a *B.t.* gene encoding a toxin into a microorganism host under conditions which allow for stable maintenance and expression of the gene. These methods are well known to those skilled in the art and are described, for example, in United States Patent No. 5,135,867, which is incorporated herein by reference.

Alternatively, a plant transformed to express a toxin of the subject invention can be used to contact the target pest with the toxin. Synthetic genes which are functionally equivalent to the novel toxins of the subject invention can also be used to transform hosts. Methods for the production of synthetic genes can be found in, for example, U.S. Patent No. 5,380,831.

Treatment of cells. As mentioned above, *B.t.* or recombinant cells expressing a *B.t.* toxin can be treated to prolong the toxin activity and stabilize the cell. The pesticide microcapsule that is formed comprises the *B.t.* toxin within a cellular structure that has been stabilized and will protect the toxin when the microcapsule is applied to the environment of the target pest. Methods for treatment of microbial cells are disclosed in United States Patent Nos. 4,695,455 and 4,695,462, which are incorporated herein by reference.

Growth of cells. The cellular host containing the *B.t.* insecticidal gene may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage, providing for a selective medium so that substantially all or all of the cells retain the *B.t.* gene. These cells may then be harvested in accordance with conventional ways. Alternatively, the cells can be treated prior to harvesting.

The *B.t.* cells of the invention can be cultured using standard art media and fermentation techniques. Upon completion of the fermentation cycle the bacteria can be harvested by first separating the *B.t.* spores and crystals from the fermentation broth by means well known in the art. Any *B.t.* spores and crystals can be recovered employing well-known techniques and used as a conventional δ-endotoxin *B.t.* preparation. For example, the spores and crystals can be formulated into a wettable powder, liquid concentrate, granules or other formulations by the addition of surfactants, dispersants, inert carriers, and other components to facilitate handling and application for particular target pests. These formulations and application procedures are all well known in the art. Alternately, the supernatant from the fermentation process can be used to obtain toxins according to the present invention. Soluble, secreted toxins are then isolated and purified employing well-known techniques.

Methods and formulations for control of pests. Control of lepidopterans using the isolates, toxins, and genes of the subject invention can be accomplished by a variety of methods known to those skilled in the art. These methods include, for example, the application of *B.t.* isolates to the pests (or their location), the application of recombinant microbes to the pests (or

their locations), and the transformation of plants with genes which encode the pesticidal toxins of the subject invention. Recombinant microbes may be, for example, a *B.t.*, *E. coli*, or *Pseudomonas*. Transformations can be made by those skilled in the art using standard techniques. Materials necessary for these transformations are disclosed herein or are otherwise readily available to the skilled artisan.

Formulated bait granules containing an attractant and toxins of the *B.t.* isolates, or recombinant microbes comprising the genes obtainable from the *B.t.* isolates disclosed herein, can be applied to the soil. Formulated product can also be applied as a seed-coating or root treatment or total plant treatment at later stages of the crop cycle. Plant and soil treatments of *B.t.* cells may be employed as liquids, wettable powders, granules, or dusts, by mixing with various inert materials, such as inorganic minerals (phyllosilicates, carbonates, sulfates, phosphates, and the like) or botanical materials (powdered corncobs, rice hulls, walnut shells, and the like).

As would be appreciated by a person skilled in the art, the pesticidal concentration will vary widely depending upon the nature of the particular formulation, particularly whether it is a concentrate or to be used directly. The pesticide will be present in at least about 1% by weight and may be 100% by weight. The dry formulations will have from about 1-95% by weight of the pesticide while the liquid formulations will generally be from about 1-60% by weight of the solids in the liquid phase. The formulations will generally have from about 10^2 to about 10^4 cells/mg. These formulations that contain cells will be administered at about 50 mg (liquid or dry) to 1 kg or more per hectare.

The formulations can be applied to the environment of the pest, *e.g.*, soil and foliage, by spraying, dusting, sprinkling, or the like.

Mutants. Mutants of novel isolates obtainable according to the invention can be made by procedures well known in the art. For example, an asporogenous mutant can be obtained through ethylmethane sulfonate (EMS) mutagenesis of an isolate. The mutants can be made using ultraviolet light and nitrosoguanidine by procedures well known in the art.

Polynucleotide probes. Hybridization may be used to test whether two pieces of DNA are complementary in their base sequences. It is this hybridization mechanism which facilitates the use of probes of the subject invention to readily detect and characterize DNA sequences of interest. The probes may be RNA or DNA. The probe will normally have at least about 10 bases, more usually at least about 18 bases, and may have up to about 50 bases or more, usually not having more than about 200 bases if the probe is made synthetically. However, longer

probes can readily be utilized, and such probes can be, for example, several kilobases in length. The probes may be labeled utilizing techniques which are well known to those skilled in this art.

One approach for the use of the subject invention as probes entails first identifying by Southern blot analysis of a gene bank of the *B.t.* isolate all DNA segments homologous with the disclosed nucleotide sequences. Thus, it is possible, without the aid of biological analysis, to know in advance the probable activity of many new *B.t.* isolates, and of the individual endotoxin gene products expressed by a given *B.t.* isolate. Such a probe analysis provides a rapid method for identifying potentially commercially valuable insecticidal endotoxin genes within the multifarious subspecies of *B.t.*

The particular hybridization technique is not essential to the subject invention. As improvements are made in hybridization techniques, they can be readily applied.

The nucleotide segments of the subject invention which are used as probes can be synthesized by use of DNA synthesizers using standard procedures. In the use of the nucleotide segments as probes, the particular probe is labeled with any suitable label known to those skilled in the art, including radioactive and non-radioactive labels.

Various degrees of stringency of hybridization can be employed. The more severe the conditions, the greater the complementarity that is required for duplex formation. Severity can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Preferably, hybridization is conducted under stringent conditions by techniques well known in the art, as described, for example, in Keller, G.H., M.M. Manak (1987) *DNA Probes*, Stockton Press, New York, NY., pp. 169-170. As used herein "stringent" conditions for hybridization refers to conditions which achieve the same, or about the same, degree of specificity of hybridization as the conditions employed by the current applicants. Specifically, hybridization of immobilized DNA on Southern blots with 32P-labeled gene-specific probes was performed by standard methods (Maniatis *et al.*). In general, hybridization and subsequent washes were carried out under stringent conditions that allowed for detection of target sequences with homology to the exemplified toxin genes. For double-stranded DNA gene probes, hybridization was carried out overnight at 20-25° C below the melting temperature (Tm) of the DNA hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula (Beltz, G.A., K.A. Jacobs, T.H. Eickbush, P.T. Cherbas, and F.C. Kafatos [1983] *Methods of Enzymology*, R. Wu, L. Grossman and K. Moldave [eds.] Academic Press, New York 100:266-285).

Tm=81.5° C+16.6 Log[Na+]+0.41(%G+C)-0.61(%formamide)-600/length of duplex in base pairs.

Washes are typically carried out as follows:

(1) Twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash).

(2) Once at Tm-20°C for 15 minutes in 0.2X SSPE, 0.1% SDS (moderate stringency wash).

For oligonucleotide probes, hybridization was carried out overnight at 10-20°C below the melting temperature (Tm) of the hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. Tm for oligonucleotide probes was determined by the following formula:

Tm (° C)=2(number T/A base pairs) +4(number G/C base pairs)

(Suggs, S.V., T. Miyake, E.H. Kawashime, M.J. Johnson, K. Itakura, and R.B. Wallace [1981] *ICN-UCLA Symp. Dev. Biol. Using Purified Genes*, D.D. Brown [ed.], Academic Press, New York, 23:683-693).

Washes were typically carried out as follows:

(1) Twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash).

(2) Once at the hybridization temperature for 15 minutes in 1X SSPE, 0.1% SDS (moderate stringency wash).

PCR technology. The DNA sequences of the subject invention can be used as primers for PCR amplification. In performing PCR amplification, a certain degree of mismatch can be tolerated between primer and template. Therefore, mutations, deletions, and insertions (especially additions of nucleotides to the 5′ end) of the exemplified primers fall within the scope of the subject invention. Mutations, insertions and deletions can be produced in a given primer by methods known to an ordinarily skilled artisan.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 – General Culturing Methods for *B.t.* Isolates Referred to Herein

A subculture of *B.t.* isolates, or mutants thereof, can be used to inoculate the following peptone, glucose, salts medium:

Bacto Peptone	7.5 g/l
Glucose	1.0 g/l

KH_2PO_4	3.4 g/l
K_2HPO_4	4.35 g/l
Salt Solution	5.0 ml/l
$CaCl_2$ Solution	5.0 ml/l
pH 7.2	

Salts Solution (100 ml)

$MgSO_4 \cdot 7H_2O$	2.46 g
$MnSO_4 \cdot H_2O$	0.04 g
$ZnSO_4 \cdot 7H_2O$	0.28 g
$FeSO_4 \cdot 7H_2O$	0.40 g

$CaCl_2$ Solution (100 ml)

$CaCl_2 \cdot 2H_2O$	3.66 g

The salts solution and $CaCl_2$ solution are filter-sterilized and added to the autoclaved and cooked broth at the time of inoculation. Flasks are incubated at 30°C on a rotary shaker at 200 rpm for 64 hr.

The above procedure can be readily scaled up to large fermentors by procedures well known in the art.

The *B.t.* toxins obtainable with the above fermentation, can be isolated by procedures well known in the art. A frequently-used procedure is to subject the harvested fermentation broth to separation techniques, *e.g.*, centrifugation.

Example 2 – Identification of Genes Encoding Novel Lepidopteran-Active *Bacillus thuringiensis* Toxins

A DNA-based polymerase chain reaction (PCR) technique was used for the identification and classification of novel toxin genes in *B.t.* strains. Two PCR primers useful for the identification of toxin genes (Forward 1 and Reverse 1) were designed. These primers contain degenerate codons in the nucleotide positions designated by ambiguity codes, and have restriction sites incorporated into the 5′ ends to enable molecular cloning of novel, amplified DNA fragments. The sequences of these oligonucleotides are:

Forward 1:

5' - GGCCACTAGT AAAAAGGAGA TAACCATGAA TAATGTATTG AATARYGGAA T - 3' (SEQ ID NO. 1)

Reverse 1:

5' - GGCCCTCGAG GGTACCCAAA CCTTAATAAA GTGGTGRAAK ATTAGTTGG - 3' (SEQ ID NO. 2)

Primers were synthesized using an Applied Biosystems model 381A DNA synthesizer. Toxin genes were then amplified from genomic *B.t.* DNA templates with these primers by standard PCR protocols (Perkin-Elmer) as follows: DNA templates for PCR were prepared from *B.t.* cells grown for 18 hours on agar plates. A loopful of cells were resuspended in TE buffer containing 50 µg/ml proteinase K and incubated at 55°C for 15 minutes. The cell suspensions were then boiled for 15 minutes. Cellular debris was pelleted in a microfuge, and the supernatant containing the DNA was transferred to a clean tube. Ten µl of this crude genomic DNA template was then used in a 100 µl PCR reaction mixture comprised of 50 mM KCl, 10 mM Tris-Cl (pH 8.3), 1.5 mM $MgCl_2$, 200 µM each dNTP, 0.1-1 µM each primer, and 2.5 units of Taq DNA polymerase.

Example 3 – Restriction Fragment Length Polymorphism (RFLP) Analysis of *Bacillus thuringiensis* Toxin Genes

PCR amplification using primer pair 1 (Forward 1 and Reverse 1)is expected to yield DNA fragments approximately 1900 base pairs in length from *B.t.* toxin genes related to the *cry*2 subfamily. Amplified gene sequences were discriminated from one another, and from known genes, by comparing the sizes of DNA restriction fragments generated by digestion of the PCR products with, for example, BglII, HincII, ScaI, or HinFI (Table 5). Briefly, approximately 0.25 - 1µg DNA from a PCR reaction was digested with a given restriction enzyme and electrophoresed on an agarose or polyacrylamide gel. The gel was then stained with ethidium bromide and DNA restriction fragments were visualized by illumination with UV light at 260-280 nm. The sizes of the restriction fragments were determined by their electrophoretic mobility relative to standard DNA fragments of known sizes. In some strains the number of fragments suggested the presence of more than one amplified toxin gene.

Table 5. Sizes of restriction fragments obtained by digestion of PCR-amplified DNA

B.t. toxin gene (GenBank Accession Number) or source strain	Restriction enzyme	Approximate DNA fragment size (base pairs)
cry2Aa1 (M31738)	*Bgl*II	616, 1333
cry2Aa1 (M31738)	*Sca*I	937, 1012
cry2Aa1 (M31738)	*Hin*FI	51, 223, 340, 363, 375, 597
cry2Ab1 (M23724)	*Hinc*II	815, 1134
cry2Ab1 (M23724)	*Hin*FI	51, 105, 112, 223, 263, 363, 832
cry2Ac (X57252)	*Sca*I	185, 1731
cry2Ac (X57252)	*Hin*FI	112, 223, 244, 293, 360, 684
PS192M4	*Bgl*II	616, 1339
PS192M4	*Hinc*II	813, 1135
PS192M4	*Sca*I	943, 1012
PS192M4	*Hin*FI	51, 112, 175, 188, 223, 261, 269, 340, 363, 597, 1161
HD573	*Hinc*II	813, 1135
HD573	*Sca*I	185, 1734
HD573	*Hin*FI	112, 223, 244, 261, 293, 360, 363, 687, 1161
HD525	*Hinc*II	813, 1135
HD525	*Sca*I	185, 1734
HD525	*Hin*FI	51, 112, 223, 244, 261, 293, 360, 363, 687, 1161
PS86I2	*Hinc*II	793, 1109
PS86I2	*Hin*FI	51, 112, 263, 341, 1135

Genes from strains with unique restriction fragment polymorphisms were cloned into pBluescript SK (Stratagene, San Diego, CA) and transformed into *E. coli* NM522 for further study. Subcultures of recombinant *E. coli* strains harboring these plasmids encoding these new toxins were deposited in the permanent collection of the Patent Culture Collection (NRRL), Regional Research Center, 1815 North University Street, Peoria, Illinois 61604 on October 17, 1996.

Example 4 – DNA Sequence Analysis of Novel Toxin Genes

DNA templates for automated sequencing were amplified by PCR using vector primers. These DNA templates were sequenced using Applied Biosystems (Foster City, CA) automated

sequencing methodologies. Novel toxin gene sequences (SEQ ID NOs. 3, 5, 7, and 9) and their respective predicted polypeptide sequences (SEQ ID NOs. 4, 6, 8, and 10) are listed in Table 6, below.

Table 6.

Source Strain	Nucleotide SEQ ID NO.	Peptide SEQ ID NO.
192M4	3	4
HD573	5	6
HD525	7	8
86I2	9	10

Example 5 – Heterologous Expression of Novel *B.t.* Toxins in *Pseudomonas fluorescens*

The toxin genes listed above were engineered into plasmid vectors by standard DNA cloning methods, and transformed into *Pseudomonas fluorescens*. Recombinant bacterial strains were grown in shake flasks for production of toxin for expression and quantitative bioassay against a variety of lepidopteran insect pests.

Example 6 – Activity of Novel *B.t.* Toxins Against *Heliothis virescens* (Fabricius) and *Helicoverpa zea* (Boddie)

Suspensions of powders containing recombinant clones according to the subject invention were prepared by individually mixing powder samples with distilled water and agitating vigorously. Suspensions were mixed with toasted soy flour artificial diet at a rate of 6 mL suspension plus 54 mL diet, yielding a concentration of 100 µg toxin/mL finished diet. After vortexing, this mixture was poured into plastic trays with compartmentalized 3 ml wells (Nutrend Container Corporation, Jacksonville, FL). A water blank containing no recombinant toxin served as the control. First instar larvae (USDA-ARS, Stoneville, MS) were placed singly into the diet mixture. Wells were then sealed with "MYLAR" sheeting (ClearLam Packaging, IL) using a tacking iron, and several pinholes were made in each well to provide gas exchange. Larvae were held at 25°C in a 14:10 (light:dark) holding room. Mortality was recorded after six days.

Table 7. *H. virescens* larval mortality with toxins in diet incorporation bioassays

Source Strain	Percent Mortality
192M4	87
HD573	80
HD525	17
water control	8

Table 8. *H. zea* larval mortality with toxins in diet incorporation bioassays

Source Strain	Percent Mortality
192M4	19
HD525	21
water control	8

Example 7 – Activity of Novel *B.t.* Toxins Against *Ostrinia nubilalis* (Huebner)

Test suspensions were prepared in 0.5 ml or 1 ml volumes by mixing powder samples with distilled water. Test suspensions were held in sterile-packaged 12 x 75 mm polypropylene tubes with snap cap (*e.g.*, Elkay Laboratory Products). Tubes were placed in hot block (*e.g.*, Fisher Scientific Hot Block) prewarmed to 34-35°C approximately 15 minutes (or less) prior to dispensation of the diet. The test suspensions were vortexed for a few seconds just prior to the addition of the diet to the 12 x 75 mm tube. To the 0.5 ml or 1 ml volumes was added 1 or 2 ml diet, respectively. The diet was measured and squirted into the tube by means of a 3 ml or 5 ml syringe with rubber tip plunger. The tube with the test suspension and diet was vortexed for 5-10 seconds or until visibly mixed. The toxin/diet suspension was then dispensed into a pre-labeled 96-well assay tray.

Diet was dispensed into the 96-well assay tray by means of a repeater pipettor with a 1.25 ml capacity pipet tip at a 4 setting for approximately 100 µl per well.

Larvae were infested one per well and sealed with waxy adhesive covering by heat treatment with iron (Oliver Products, MI). Bioassays were held at 26-28°C, and data were collected in 7 days.

Table 9. *O. nubilalis* larval mortality with toxins in diet incorporation bioassays

Source Strain	Percent Mortality
192M4	50
HD573	90
water control	8

Example 8 – Insertion of Toxin Genes Into Plants

One aspect of the subject invention is the transformation of plants with genes encoding the insecticidal toxin of the subject invention. The transformed plants are resistant to attack by the target pest.

Genes encoding pesticidal toxins, as disclosed herein, can be inserted into plant cells using a variety of techniques which are well known in the art. For example, a large number of cloning vectors comprising a replication system in *E. coli* and a marker that permits selection of the transformed cells are available for preparation for the insertion of foreign genes into higher plants. The vectors comprise, for example, pBR322, pUC series, M13mp series, pACYC184, etc. Accordingly, the sequence encoding the *B.t.* toxin can be inserted into the vector at a suitable restriction site. The resulting plasmid is used for transformation into *E. coli*. The *E. coli* cells are cultivated in a suitable nutrient medium, then harvested and lysed. The plasmid is recovered. Sequence analysis, restriction analysis, electrophoresis, and other biochemical-molecular biological methods are generally carried out as methods of analysis. After each manipulation, the DNA sequence used can be cleaved and joined to the next DNA sequence. Each plasmid sequence can be cloned in the same or other plasmids. Depending on the method of inserting desired genes into the plant, other DNA sequences may be necessary. If, for example, the Ti or Ri plasmid is used for the transformation of the plant cell, then at least the right border, but often the right and the left border of the Ti or Ri plasmid T-DNA, has to be joined as the flanking region of the genes to be inserted.

The use of T-DNA for the transformation of plant cells has been intensively researched and sufficiently described in EP 120 516; Hoekema (1985) In: *The Binary Plant Vector System*, Offset-durkkerij Kanters B.V., Alblasserdam, Chapter 5; Fraley *et al.*, *Crit. Rev. Plant Sci.* 4:1-46; and An *et al.* (1985) *EMBO J.* 4:277-287.

Once the inserted DNA has been integrated in the genome, it is relatively stable there and, as a rule, does not come out again. It normally contains a selection marker that confers on

the transformed plant cells resistance to a biocide or an antibiotic, such as kanamycin, G 418, bleomycin, hygromycin, or chloramphenicol, *inter alia*. The individually employed marker should accordingly permit the selection of transformed cells rather than cells that do not contain the inserted DNA.

A large number of techniques are available for inserting DNA into a plant host cell. Those techniques include transformation with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as transformation agent, fusion, injection, biolistics (microparticle bombardment), or electroporation as well as other possible methods. If Agrobacteria are used for the transformation, the DNA to be inserted has to be cloned into special plasmids, namely either into an intermediate vector or into a binary vector. The intermediate vectors can be integrated into the Ti or Ri plasmid by homologous recombination owing to sequences that are homologous to sequences in the T-DNA. The Ti or Ri plasmid also comprises the vir region necessary for the transfer of the T-DNA. Intermediate vectors cannot replicate themselves in Agrobacteria. The intermediate vector can be transferred into *Agrobacterium tumefaciens* by means of a helper plasmid (conjugation). Binary vectors can replicate themselves both in *E. coli* and in Agrobacteria. They comprise a selection marker gene and a linker or polylinker which are framed by the right and left T-DNA border regions. They can be transformed directly into Agrobacteria (Holsters *et al.* [1978] *Mol. Gen. Genet.* 163:181-187). The *Agrobacterium* used as host cell is to comprise a plasmid carrying a vir region. The vir region is necessary for the transfer of the T-DNA into the plant cell. Additional T-DNA may be contained. The bacterium so transformed is used for the transformation of plant cells. Plant explants can advantageously be cultivated with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* for the transfer of the DNA into the plant cell. Whole plants can then be regenerated from the infected plant material (for example, pieces of leaf, segments of stalk, meristematic tissue, roots, but also protoplasts or suspension-cultivated cells) in a suitable medium, which may contain antibiotics or biocides for selection. The plants so obtained can then be tested for the presence of the inserted DNA. No special demands are made of the plasmids in the case of injection and electroporation. It is possible to use ordinary plasmids, such as, for example, pUC derivatives. In biolistics transformation, plasmid DNA or linear DNA can be employed.

The transformed cells are regenerated into morphologically normal plants in the usual manner. If a transformation event involves a germ line cell, the inserted DNA and corresponding phenotypic trait(s) will be transmitted to progeny plants. Such plants can be grown in the normal manner and crossed with plants that have the same transformed hereditary

factors or other hereditary factors. The resulting hybrid individuals have the corresponding phenotypic properties.

In a preferred embodiment of the subject invention, plants will be transformed with genes wherein the codon usage has been optimized for plants. See, for example, U.S. Patent No. 5,380,831. Also, advantageously, plants encoding a truncated toxin will be used. The truncated toxin typically will encode about 55% to about 80% of the full length toxin. Methods for creating synthetic *B.t.* genes for use in plants are known in the art.

All of the U.S. patents cited herein are hereby incorporated by reference.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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Telex number:

(ii) TITLE OF INVENTION: Toxins Active Against Pests

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: Gainesville
(D) STATE: Florida
(E) COUNTRY: USA
(F) ZIP: 32606

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Sanders, Jay M.
(B) REGISTRATION NUMBER: 39,355
(C) REFERENCE/DOCKET NUMBER: MA-709

(ix) TELECOMMUNICATION INFORMATION:
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(B) TELEFAX: (352) 372-5800

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGCCACTAGT AAAAAGGAGA TAACCATGAA TAATGTATTG AATARYGGAA T 41

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGCCCTCGAG GGTACCCAAA CCTTAATAAA GTGGTGRAAK ATTAGTTGG 49

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1908 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGAATAATG TATTGAATAG TGGAAGAACA ACTATTTGTA ATGCGTATAA TGTAGTGGCT 60

CACGATCCAT TTAGTTTTGA ACATAAATCA TTAGATACCA TCCAAGAAGA ATGGATGGAG 120

TGGAAAAGAA CAGATCATAG TTTATATGTA GCTCCTGTAG TCGGAACTGT GTCTAGTTTT 180

CTGCTAAAGA AAGTGGGGAG TCTAATTGGA AAAAGGATAT TGAGTGAATT ATGGGGGTTA 240

ATATTTCCTA GTGGTAGTAC AAATCTAATG CAAGATATTT TAAGAGAGAC AGAACAATTC 300

CTAAATCAAA GACTTAATAC AGACACCCTT GATCGTGTAA ATGCAGAATT GGAAGGGCTC 360

CAAGCGAATA TAAGGGAGTT TAATCAACAA GTAGATAATT TTTTAAACCC TACTCAAAAC 420

CCTGTTCCTT TATCAATAAC TTCTTCAGTT AATACAATGC AGCAATTATT TCTAAATAGA 480

TTACCCCAGT TCCAGATACA AGGATACCAG TTGTTATTAT TACCTTTATT TGCACAGGCA 540

GCCAATATGC ATCTTTCTTT TATTAGAGAT GTTATTCTTA ATGCAGATGA ATGGGGCATT 600

TCAGCAGCAA CACTACGTAC GTATCGAGAC TACCTGAGAA ATTATACAAG AGATTATTCT 660

AATTATTGTA TAAATACGTA TCAAACTGCG TTTAGAGGGT TAAACACCCG TTTACACGAT 720

ATGTTAGAAT TTAGAACATA TATGTTTTTA AATGTATTTG AATATGTATC CATTTGGTCA 780

TTGTTTAAAT ATCAGAGTCT TATGGTATCT TCTGGCGCTA ATTTATATGC TAGTGGTAGT 840

GGACCACAGC AGACACAATC ATTTACTGCA CAAAACTGGC CATTTTTATA TTCTCTTTTC 900

CAAGTTAATT CGAATTATAT ATTATCTGGT ATTAGTGGTA ATAGGCTTTC TACTACCTTC 960

CCTAATATTG GTGGTTTACC GGGTAGTACT ACAATTCATT CATTGAACAG TGCCAGGGTT 1020

AATTATAGCG GAGGAGTTTC ATCTGGTCTC ATAGGGGCGA CTAATCTCAA TCACAACTTT 1080

AATTGCAGCA CGGTCCTCCC TCCTTTATCA ACACCATTTG TTAGAAGTTG GCTGGATTCA 1140

GGTACAGATC GAGAGGGCGT TGCTACCTCT ACGACTTGGC AGACAGAATC CTTCCAAATA 1200

ACTTCAGGTT TAAGGTGTGG TGCTTTTCCT TTTTCAGCTC GTGGAAATTC AAACTATTTC 1260

CCAGATTATT TTATCCGTAA TATTTCTGGG GTTCCTTTAG TTATTAGAAA CGAAGATCTA 1320

ACAAGACCGT TACACTATAA CCAAATAAGA AATATAGAAA GTCCTTCGGG AACACCTGGT 1380

GGATTACGAG CTTATATGGT ATCTGTGCAT AACAGAAAAA ATAATATCTA TGCCGCTCAT 1440

GAAAATGGTA CTATGATTCA TTTGGCACCG GAAGATTATA CAGGATTTAC TATATCACCA 1500

ATACATGCCA CTCAAGTGAA TAATCAAACT CGAACATTTA TTTCTGAAAA ATTTGGAAAT 1560

CAAGGTGATT CCTTAAGATT TGAACAAAGT AACACGACAG CTCGTTATAC GCTTAGAGGG 1620

AATGGAAATA GTTACAATCT TTATTTAAGG GTATCTTCTC TAGGAAATTC CACTATTCGA 1680

GTTACTATAA ACGGAAGAGT TTATACTGTT CCAAATGTTA ATACAAATAT AAATAACGAT 1740

GGAGTCATTG ATAATGGAGC TCGTTTTTCA GATATTAATA TCGGTAATGT AGTAGCAAGT 1800

GATAATACTA ATGTACCGTT AGATATAAAC GGGACATTAA GTTCTGGAAC TCAATTTGAG 1860

CTTATGAATA TTATGTTTGT TCCAACTAAT CTTCCACCAC TTTATTAA 1908

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 635 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asn Asn Val Leu Asn Ser Gly Arg Thr Thr Ile Cys Asn Ala Tyr
1 5 10 15

Asn Val Val Ala His Asp Pro Phe Ser Phe Glu His Lys Ser Leu Asp
20 25 30

Thr Ile Gln Glu Glu Trp Met Glu Trp Lys Arg Thr Asp His Ser Leu
35 40 45

Tyr Val Ala Pro Val Val Gly Thr Val Ser Ser Phe Leu Leu Lys Lys
50 55 60

Val Gly Ser Leu Ile Gly Lys Arg Ile Leu Ser Glu Leu Trp Gly Leu
65 70 75 80

Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Gln Asp Ile Leu Arg Glu
85 90 95

Thr Glu Gln Phe Leu Asn Gln Arg Leu Asn Thr Asp Thr Leu Asp Arg
100 105 110

Val Asn Ala Glu Leu Glu Gly Leu Gln Ala Asn Ile Arg Glu Phe Asn
115 120 125

Gln Gln Val Asp Asn Phe Leu Asn Pro Thr Gln Asn Pro Val Pro Leu
130 135 140

Ser Ile Thr Ser Ser Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg
145 150 155 160

Leu Pro Gln Phe Gln Ile Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu
165 170 175

Phe Ala Gln Ala Ala Asn Met His Leu Ser Phe Ile Arg Asp Val Ile
180 185 190

Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr
195 200 205

Arg Asp Tyr Leu Arg Asn Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile
210 215 220

Asn Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp
225 230 235 240

Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val
245 250 255

Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Met Val Ser Ser Gly
260 265 270

Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Gln Gln Thr Gln Ser Phe
275 280 285

Thr Ala Gln Asn Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser
290 295 300

Asn Tyr Ile Leu Ser Gly Ile Ser Gly Asn Arg Leu Ser Thr Thr Phe
305 310 315 320

Pro Asn Ile Gly Gly Leu Pro Gly Ser Thr Thr Ile His Ser Leu Asn
325 330 335

Ser Ala Arg Val Asn Tyr Ser Gly Gly Val Ser Ser Gly Leu Ile Gly
340 345 350

Ala Thr Asn Leu Asn His Asn Phe Asn Cys Ser Thr Val Leu Pro Pro
355 360 365

Leu Ser Thr Pro Phe Val Arg Ser Trp Leu Asp Ser Gly Thr Asp Arg
370 375 380

Glu Gly Val Ala Thr Ser Thr Thr Trp Gln Thr Glu Ser Phe Gln Ile
385 390 395 400

Thr Ser Gly Leu Arg Cys Gly Ala Phe Pro Phe Ser Ala Arg Gly Asn
405 410 415

Ser Asn Tyr Phe Pro Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Pro
420 425 430

Leu Val Ile Arg Asn Glu Asp Leu Thr Arg Pro Leu His Tyr Asn Gln
435 440 445

Ile Arg Asn Ile Glu Ser Pro Ser Gly Thr Pro Gly Gly Leu Arg Ala
450 455 460

Tyr Met Val Ser Val His Asn Arg Lys Asn Asn Ile Tyr Ala Ala His
465 470 475 480

Glu Asn Gly Thr Met Ile His Leu Ala Pro Glu Asp Tyr Thr Gly Phe
485 490 495

Thr Ile Ser Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr
500 505 510

Phe Ile Ser Glu Lys Phe Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu
515 520 525

Gln Ser Asn Thr Thr Ala Arg Tyr Thr Leu Arg Gly Asn Gly Asn Ser
530 535 540

Tyr Asn Leu Tyr Leu Arg Val Ser Ser Leu Gly Asn Ser Thr Ile Arg
545 550 555 560

Val Thr Ile Asn Gly Arg Val Tyr Thr Val Pro Asn Val Asn Thr Asn
565 570 575

Ile Asn Asn Asp Gly Val Ile Asp Asn Gly Ala Arg Phe Ser Asp Ile
580 585 590

Asn Ile Gly Asn Val Val Ala Ser Asp Asn Thr Asn Val Pro Leu Asp
595 600 605

Ile Asn Gly Thr Leu Ser Ser Gly Thr Gln Phe Glu Leu Met Asn Ile
610 615 620

Met Phe Val Pro Thr Asn Leu Pro Pro Leu Tyr
625 630 635

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1872 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGAATAATG TATTGAATAG CGGAAGAAAT ACTACTTGTC ATGCACATAA TGTAGTTGCT 60

CATGATCCAT TTAGTTTTGA ACATAAATCA TTAAATACCA TAGAAAAAGA ATGGAAAGAA 120

TGGAAAAGAA CTGATCATAG TTTATATGTA GCCCCTATTG TGGGAACTGT GGGTAGTTTT 180

CTATTAAAGA AAGTAGGGAG TCTTGTTGGA AAAAGGATAC TGAGTGAGTT ACAGAATTTA 240

ATTTTTCCTA GTGGTAGTAT AGATTTAATG CAAGAGATTT TAAGAGCGAC AGAACAATTC 300

ATAAATCAAA GGCTTAATGC AGACACCCTT GGTCGTGTAA ATGCAGAATT GGCAGGTCTT 360

CAAGCGAATG TGGCAGAGTT TAATCGACAA GTAGATAATT TTTTAAACCC TAATCAAAAC 420

CCTGTTCCTT TAGCAATAAT TGATTCAGTT AATACATTGC AGCAATTATT TCTAAGTAGA 480

TTACCACAGT TCCAGATACA AGGCTATCAA CTGTTATTAT TACCTTTATT TGCACAGGCA 540

GCCAATTTAC ATCTTTCTTT TATTAGAGAT GTCATCCTTA ATGCAGATGA ATGGGGCATT 600

TCAGCAGCAA CAGTACGCAC ATATAGAGAT CACCTGAGAA ATTTCACAAG AGATTACTCT 660

AATTATTGTA TAAATACGTA TCAAACTGCA TTTAGAGGTT TAAACACTCG TTTACACGAT 720

ATGTTAGAAT TTAGAACATA TATGTTTTTA AATGTATTTG AATATGTCTC TATCTGGTCG 780

TTATTTAAAT ATCAAAGCCT TCTAGTATCT TCCGGCGCTA ATTTATATGC GAGTGGTAGT 840

GGTCCAACAC AATCATTTAC AGCACATAAC TGGCCATTTT TATATTCTCT TTTCCAAGTT 900

AATTCTAATT ATGTATTAAA TGGTTTGAGT GGTGCTAGGA CCACCATTAC TTTCTCTAAT 960

ATTGGTGGTC TTCCCGGTTC TACCACAACT CAAACATTGC ATTTTGCGAG GATTAATTAT 1020

AGAGGTGGAG TGTCATCTAG CCGCATAGGT CAAGCTAATC TTAATCAAAA CTTTAACATT 1080

TCCACACTTT TCAATCCTTT ACAAACACCG TTTATTAGAA GTTGGCTAGA TTCTGGTACA 1140

GATCGGGAGG GCGTTGCCAC CTCTACAAAC TGGCAATCAG GAGCCTTTGA GACAACTTTA 1200

TTACGATTTA GCATTTTTTC AGCTCGTGGT AATTCGAACT TTTTCCCAGA TTATTTTATC 1260

CGTAATATTT CTGGTGTTGT TGGGACTATT AGCAACGCAG ATTTAGCAAG ACCTCTACAC 1320

TTTAATGAAA TAAGAGATAT AGGAACGACA GCAGTCGCTA GCCTTGTAAC AGTGCATAAC 1380

AGAAAAAATA ATATCTATGA CACTCATGAA AATGGTACTA TGATTCATTT AGCGCCAAAT 1440

GACTATACAG GATTTACCGT ATCTCCAATA CATGCCACTC AAGTAAATAA TCAAATTCGA 1500

ACGTTTATTT CCGAAAAATA TGGTAATCAG GGTGATTCCT TGAGATTTGA GCTAAGCAAC 1560

ACAACGGCTC GATACACACT TAGAGGGAAT GGAAATAGTT ACAATCTTTA TTTAAGAGTA 1620

TCTTCAATAG GAAGTTCCAC AATTCGAGTT ACTATAAACG GTAGAGTTTA TACTGCAAAT 1680

GTTAATACTA CCACAAATAA TGATGGAGTA CTTGATAATG GAGCTCGTTT TTCAGATATT 1740

AATATCGGTA ATGTAGTGGC AAGTGCTAAT ACTAATGTAC CATTAGATAT ACAAGTGACA 1800

TTTAACGGCA ATCCACAATT TGAGCTTATG AATATTATGT TTGTTCCAAC TAATCCTTCA 1860

CCACTTTATT AA 1872

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 623 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Asn Asn Val Leu Asn Ser Gly Arg Asn Thr Thr Cys His Ala His
1 5 10 15

Asn Val Val Ala His Asp Pro Phe Ser Phe Glu His Lys Ser Leu Asn
20 25 30

Thr Ile Glu Lys Glu Trp Lys Glu Trp Lys Arg Thr Asp His Ser Leu
35 40 45

Tyr Val Ala Pro Ile Val Gly Thr Val Gly Ser Phe Leu Leu Lys Lys
50 55 60

Val Gly Ser Leu Val Gly Lys Arg Ile Leu Ser Glu Leu Gln Asn Leu
65 70 75 80

Ile Phe Pro Ser Gly Ser Ile Asp Leu Met Gln Glu Ile Leu Arg Ala
85 90 95

Thr Glu Gln Phe Ile Asn Gln Arg Leu Asn Ala Asp Thr Leu Gly Arg
100 105 110

Val Asn Ala Glu Leu Ala Gly Leu Gln Ala Asn Val Ala Glu Phe Asn
115 120 125

Arg Gln Val Asp Asn Phe Leu Asn Pro Asn Gln Asn Pro Val Pro Leu
130 135 140

Ala Ile Ile Asp Ser Val Asn Thr Leu Gln Gln Leu Phe Leu Ser Arg
145 150 155 160

Leu Pro Gln Phe Gln Ile Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu
165 170 175

Phe Ala Gln Ala Ala Asn Leu His Leu Ser Phe Ile Arg Asp Val Ile
180 185 190

Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Val Arg Thr Tyr
195 200 205

Arg Asp His Leu Arg Asn Phe Thr Arg Asp Tyr Ser Asn Tyr Cys Ile
210 215 220

Asn Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp
225 230 235 240

Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val
245 250 255

Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Leu Val Ser Ser Gly
260 265 270

Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Thr Gln Ser Phe Thr Ala
275 280 285

His Asn Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser Asn Tyr
290 295 300

Val Leu Asn Gly Leu Ser Gly Ala Arg Thr Thr Ile Thr Phe Ser Asn
305 310 315 320

Ile Gly Gly Leu Pro Gly Ser Thr Thr Thr Gln Thr Leu His Phe Ala
325 330 335

Arg Ile Asn Tyr Arg Gly Gly Val Ser Ser Ser Arg Ile Gly Gln Ala
340 345 350

Asn Leu Asn Gln Asn Phe Asn Ile Ser Thr Leu Phe Asn Pro Leu Gln
355 360 365

Thr Pro Phe Ile Arg Ser Trp Leu Asp Ser Gly Thr Asp Arg Glu Gly
370 375 380

Val Ala Thr Ser Thr Asn Trp Gln Ser Gly Ala Phe Glu Thr Thr Leu
385 390 395 400

Leu Arg Phe Ser Ile Phe Ser Ala Arg Gly Asn Ser Asn Phe Phe Pro
405 410 415

Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Val Gly Thr Ile Ser Asn
420 425 430

Ala Asp Leu Ala Arg Pro Leu His Phe Asn Glu Ile Arg Asp Ile Gly
435 440 445

Thr Thr Ala Val Ala Ser Leu Val Thr Val His Asn Arg Lys Asn Asn
450 455 460

Ile Tyr Asp Thr His Glu Asn Gly Thr Met Ile His Leu Ala Pro Asn
465 470 475 480

Asp Tyr Thr Gly Phe Thr Val Ser Pro Ile His Ala Thr Gln Val Asn
485 490 495

Asn Gln Ile Arg Thr Phe Ile Ser Glu Lys Tyr Gly Asn Gln Gly Asp
500 505 510

Ser Leu Arg Phe Glu Leu Ser Asn Thr Thr Ala Arg Tyr Thr Leu Arg
515 520 525

Gly Asn Gly Asn Ser Tyr Asn Leu Tyr Leu Arg Val Ser Ser Ile Gly
530 535 540

Ser Ser Thr Ile Arg Val Thr Ile Asn Gly Arg Val Tyr Thr Ala Asn
545 550 555 560

Val Asn Thr Thr Thr Asn Asn Asp Gly Val Leu Asp Asn Gly Ala Arg
565 570 575

Phe Ser Asp Ile Asn Ile Gly Asn Val Val Ala Ser Ala Asn Thr Asn
580 585 590

Val Pro Leu Asp Ile Gln Val Thr Phe Asn Gly Asn Pro Gln Phe Glu
595 600 605

Leu Met Asn Ile Met Phe Val Pro Thr Asn Pro Ser Pro Leu Tyr
610 615 620

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1902 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGAATAATG TATTGAATAG TGGAAGAAAT ACTATTTGTG ATGCGTATAA TGTAGTGGTT 60

CATGATCCAT TTAGTTTTCA ACATAAATCA TTAGATACCA TACAAAAAGA ATGGATGGAG 120

TGGAAAAAAG ATAATCATAG TTTATATGTA GATCCTATTG TTGGAACTGT GGCTAGTTTT 180

CTGTTAAAGA AATTGGGGAG CCTTATTGGA AAACGGATAC TGAGTGAATT ACGGAATTTA 240

ATATTTCCTA GTGGCAGTAC AAATCTAATG GAAGATATTT TAAGAGAGAC AGAAAAATTC 300

CTAAATCAAA AACTTAATAC AGACACTCTT TCCCGTGTAA ATGCGGAATT GACAGGGCTG 360

CAAGCAAATG TAGAAGAGTT TAATCGACAA GTAGATAATT TTTTGAACCC TAACCGAAAC 420

GCTGTTCCTT TATCAATAAC TTCTTCAGTT AATACAATGC AGCAATTATT TCTAAATAGA 480

TTATCCCAGT TCCAGATGCA AGGATACCAA CTGTTATTAT TACCTTTATT TGCACAGGCA 540

GCCAATTTAC ATCTTTCTTT TATTAGAGAT GTTATTCTTA ATGCAGAAGA ATGGGGCATT 600

TCAGCAGCAA CATTACGTAC GTATCAAAAT CACCTGAGAA ATTATACAAG AGATTACTCT 660

AATTATTGTA TAGATACGTA TCAAACTGCG TTTAGAGGTT TAAACACCCG TTTACACGAT 720

ATGTTAGAAT TTAGAACATA TATGTTTTTA AATGTATTTG AATATGTATC TATCTGGTCG 780

TTGTTTAAAT ATCAAAGTCT TCTAGTATCT TCTGGCGCTA ATTTATATGC AAGTGGTAGT 840

GGACCACAGC AGACCCAATT ATTTACTTCA CAAGACTGGC CATTTTTATA TTCTCTTTTC 900

CAAGTTAATT CGAATTATGT ATTATCCGGC TTTAGTGGGG CTAGTCTTTT TACTACCTTT 960

CCTAATATTG GTGGCTTACC TGGTTCTACT ACAACTCAAG CATTACTTGC TGCAAGGGTT 1020

AATTATAGTG GAGGAATTAC ATCTGGTAGT ATAGGGGGTT CTAATTTTAA TCAAAATTTT 1080

AATTGCAACA CGATATCGCC ACCTTTGTCA ACGTCATTTG TTAGAATTTG GCTAGATTCG 1140

GGTTCAGATC GACAGGGCGT TACTACCGTT ACAAATTGGC AAACAGAGTC CTTTGAGACA 1200

ACTTCAGGTT TAAGGTGTGG TGCTTTTACA CCTCGTGGTA ATTCGAACTA TTACCCTGGT 1260

TATTTTATCC GTAATATTTC TGGTGTTTCT TTAGTTCTTA GAAATGAAGA CTTAAAAAGA 1320

CCGTTATACT ATAACGAAAA AAGGAATATA GAAAGCCCTT CAGGAACACC TGGTGGAGCA 1380

AGAGCTTATA TGGTATCTGT GCATAACAAA AAAAATAACA TTTATGCAGT TCATGAAAAT 1440

GGTACTATGA TTCATTTAGC GCCGGAAGAT AATACAGGAT TTACTATATC ACCGATACAT 1500

GCCACTCAAG TGAATAATCA AACGCGAACA TTTATTTCCG AAAAATTTGG AAATCAAAGT 1560

GATTCCTTAA GATTTGAACA AAGCAACACG ACAGCTCGTT ATACCCTTAG AGGGAATGGA 1620

AATAGTTACA ATCTTTATTT AAGAGTATCT TCAATAGGAA ATTCCACTAT TCGAGTTACT 1680

ATAAACGGTA GAGTTTATAC TGCTTCAAAT GTTAATACTA CTACAAATAA CGATGGAGTT 1740

AATGATAACG GAGCTCGTTT TTCAGATATT AATATCGGTA ATGTAGTAGC AAGTAGTAAT 1800

TCTGATGTAC CATTAGATAT AAATGTAACA TTAAACTCCG GTACTCAATT TGATCTTATG 1860

AATATTATGC TTGTACCAAC TAATCTTCCA CCACTTTATT AA 1902

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 633 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Asn Asn Val Leu Asn Ser Gly Arg Asn Thr Ile Cys Asp Ala Tyr
1 5 10 15

Asn Val Val Val His Asp Pro Phe Ser Phe Gln His Lys Ser Leu Asp
20 25 30

Thr Ile Gln Lys Glu Trp Met Glu Trp Lys Lys Asp Asn His Ser Leu
35 40 45

Tyr Val Asp Pro Ile Val Gly Thr Val Ala Ser Phe Leu Leu Lys Lys
50 55 60

Leu Gly Ser Leu Ile Gly Lys Arg Ile Leu Ser Glu Leu Arg Asn Leu
65 70 75 80

Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Glu Asp Ile Leu Arg Glu
85 90 95

Thr Glu Lys Phe Leu Asn Gln Lys Leu Asn Thr Asp Thr Leu Ser Arg
100 105 110

Val Asn Ala Glu Leu Thr Gly Leu Gln Ala Asn Val Glu Glu Phe Asn
115 120 125

Arg Gln Val Asp Asn Phe Leu Asn Pro Asn Arg Asn Ala Val Pro Leu
130 135 140

Ser Ile Thr Ser Ser Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg
145 150 155 160

Leu Ser Gln Phe Gln Met Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu
165 170 175

Phe Ala Gln Ala Ala Asn Leu His Leu Ser Phe Ile Arg Asp Val Ile
180 185 190

Leu Asn Ala Glu Glu Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr
195 200 205

Gln Asn His Leu Arg Asn Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile
210 215 220

Asp Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp
225 230 235 240

Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val
245 250 255

Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Leu Val Ser Ser Gly
260 265 270

Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Gln Gln Thr Gln Leu Phe
275 280 285

Thr Ser Gln Asp Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser
290 295 300

Asn Tyr Val Leu Ser Gly Phe Ser Gly Ala Ser Leu Phe Thr Thr Phe
305 310 315 320

Pro Asn Ile Gly Gly Leu Pro Gly Ser Thr Thr Thr Gln Ala Leu Leu
325 330 335

Ala Ala Arg Val Asn Tyr Ser Gly Gly Ile Thr Ser Gly Ser Ile Gly
340 345 350

Gly Ser Asn Phe Asn Gln Asn Phe Asn Cys Asn Thr Ile Ser Pro Pro
355 360 365

Leu Ser Thr Ser Phe Val Arg Ile Trp Leu Asp Ser Gly Ser Asp Arg
370 375 380

Gln Gly Val Thr Thr Val Thr Asn Trp Gln Thr Glu Ser Phe Glu Thr
385 390 395 400

Thr Ser Gly Leu Arg Cys Gly Ala Phe Thr Pro Arg Gly Asn Ser Asn
405 410 415

Tyr Tyr Pro Gly Tyr Phe Ile Arg Asn Ile Ser Gly Val Ser Leu Val
420 425 430

Leu Arg Asn Glu Asp Leu Lys Arg Pro Leu Tyr Tyr Asn Glu Lys Arg
435 440 445

Asn Ile Glu Ser Pro Ser Gly Thr Pro Gly Gly Ala Arg Ala Tyr Met
450 455 460

Val Ser Val His Asn Lys Lys Asn Asn Ile Tyr Ala Val His Glu Asn
465 470 475 480

Gly Thr Met Ile His Leu Ala Pro Glu Asp Asn Thr Gly Phe Thr Ile
485 490 495

Ser Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr Phe Ile
500 505 510

Ser Glu Lys Phe Gly Asn Gln Ser Asp Ser Leu Arg Phe Glu Gln Ser
515 520 525

Asn Thr Thr Ala Arg Tyr Thr Leu Arg Gly Asn Gly Asn Ser Tyr Asn
530 535 540

Leu Tyr Leu Arg Val Ser Ser Ile Gly Asn Ser Thr Ile Arg Val Thr
545 550 555 560

Ile Asn Gly Arg Val Tyr Thr Ala Ser Asn Val Asn Thr Thr Thr Asn
565 570 575

Asn Asp Gly Val Asn Asp Asn Gly Ala Arg Phe Ser Asp Ile Asn Ile
580 585 590

Gly Asn Val Val Ala Ser Ser Asn Ser Asp Val Pro Leu Asp Ile Asn
595 600 605

Val Thr Leu Asn Ser Gly Thr Gln Phe Asp Leu Met Asn Ile Met Leu
610 615 620

Val Pro Thr Asn Leu Pro Pro Leu Tyr
625 630

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1902 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGAATAATG TATTGAATAA TGGAAGAAAT ACTATTTGTG ATGCGTATAA TGTAGTGGTT 60

CATGATCCAT TTAGTTTTCA ACATAAATCA TTAGATACCA TACAAAAAGA ATGGATGGAG 120

TGGAAAAAAG ATAATCATAG TTTATATGTA GATCCTATTG TTGGAACTGT GGCTAGTTTT 180

CTGTTAAAGA AATTGGGGAG CCTTATTGGA AAACGGATAC TGAGTGAATT ACGGAATTTA 240

ATATTTCCTA GTGGCAGTAC AAATCTAATG GAAGATATTT TAAGAGAGAC AGAAAAATTC 300

CTAAATCAAA AACTTAATAC AGACACTCTT TCCCGTGTAA ATGCGGAATT GACAGGGCTG 360

CAAGCAAATG TAGAAGAGTT TAATCGACAA GTAGATAATT TTTTGAACCC TAACCGAAAC 420

GCTGTTCCTT TATCAATAAC TTCTTCAGTT AATACAATGC AGCAATTATT TCTAAATAGA 480

TTATCCCAGT TCCAGATGCA AGGATACCAA CTGTTATTAT TACCTTTATT TGCACAGGCA 540

GCCAATATAC ATCTTTCTTA TATTAGAGAT GTTATTCTTA ATGCAGAAGA ATGGGGCATT 600

TCAGCAGCAA CATTACGTAC GTATCAAAAT CACCTGAGAA ATTATACAAG AGATTACTCT 660

AATTATTGTA TAGATACGTA TCAAACTGCG TTTAGAGGTT TAAACACCCG TATACACGAT 720

ATGTTAGAAT TTAGAACATA TATGTTTTTA AATGTATTTG AATATGTATC TATCTGGTCG 780

TTGTTTAAAT ATCAAAGTCT TCTAGTATCT TCTGGCGCTA ATTTATATGC AAGTGGTAGT 840

GGACCACAGC AGACCCAATT ATTTACTTCA CAAGACTGGC CATTTTTATA TTCTCTTTTC 900

CAAGTTAATT CGAATTATGT ATTATCCGGC TTTAGTGGGG CTAGTCTTTT TACTACCTTT 960

CCTAATATTG GTGGCTTACC TGGTTCTACT ACAACTCAAG CATTACTTGC TGCAAGGGTT 1020

AATTATAGTG GAGGAATTAC ATCTGGTAGT ATAGGGGGTT CTAATTTTAA TCAAAATTTT 1080

AATTGCAACA CGATATCGCC ACCTTTGTCA ACGTCATTTG TTAGAAGTTG GCTAGATTCG 1140

GGTTCAGATC GACAGGGCGT TACTACCGTT ACAAATTGGC AAACAGAGTC CTTTGAGACA 1200

ACTTCAGGTT TAAGGTGTGG TGCTTTTACA CCTCGTGGTA ATTCGAACTA TTACCCTGGT 1260

TATTTTATCC GTAATATTTC TGGTGTTTCT TTAGTTCTTA GAAATGAAGA CTTAAAAAGA 1320

CCGTTATACT ATAACGAAAA AAGGAATATA GAAAGCCCTT CAGGAACACC TGGTGGAGCA 1380

AGAGCTTATA TGGTATCTGT GCATAACAAA AAAAATAACA TTTATGCAGT TCATGAAAAT 1440

GGTACTATGA TTCATTTAGC GCCGGAAGAT AATACAGGAT TTACTATATC ACCGATACAT 1500

GCCACTCAAG TGAATAATCA AACGCGAACA TTTATTTCCG AAAAATTTGG AAATCAAGGT 1560

GATTCCTTAA GATTTGAACA AAGCAACACG ACAGCTCGTT ATACCCTTAG AGGGAATGGA 1620

AATAGTTACA ATCTTTATTT AAGAGTATCT TCAATAGGAA ATTCCACTAT TCGAGTTACT 1680

ATAAACGGTA GAGTTTATAC TGCTTCAAAT GTTAATACTA CTACAAATAA CGATGGAGTT 1740

AATGATAACG GAGCTCGTTT TTCAGATATT AATATCGGTA ATGTAGTAGC AAGTAGTAAT 1800

TCTGATGTAC CATTAGATAT AAATGTAACA TTAAACTCCG GTACTCAATT TGATCTTATG 1860

AATATTATGC TTGTACCAAC TAATATTTCA CCACTTTATT AA 1902

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 633 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Asn Asn Val Leu Asn Asn Gly Arg Asn Thr Ile Cys Asp Ala Tyr
1 5 10 15

Asn Val Val Val His Asp Pro Phe Ser Phe Gln His Lys Ser Leu Asp
20 25 30

Thr Ile Gln Lys Glu Trp Met Glu Trp Lys Lys Asp Asn His Ser Leu
35 40 45

Tyr Val Asp Pro Ile Val Gly Thr Val Ala Ser Phe Leu Leu Lys Lys
50 55 60

Leu Gly Ser Leu Ile Gly Lys Arg Ile Leu Ser Glu Leu Arg Asn Leu
65 70 75 80

Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Glu Asp Ile Leu Arg Glu
85 90 95

Thr Glu Lys Phe Leu Asn Gln Lys Leu Asn Thr Asp Thr Leu Ser Arg
100 105 110

Val Asn Ala Glu Leu Thr Gly Leu Gln Ala Asn Val Glu Glu Phe Asn
115 120 125

Arg Gln Val Asp Asn Phe Leu Asn Pro Asn Arg Asn Ala Val Pro Leu
130 135 140

Ser Ile Thr Ser Ser Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg
145 150 155 160

Leu Ser Gln Phe Gln Met Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu
165 170 175

Phe Ala Gln Ala Ala Asn Ile His Leu Ser Tyr Ile Arg Asp Val Ile
180 185 190

Leu Asn Ala Glu Glu Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr
195 200 205

Gln Asn His Leu Arg Asn Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile
210 215 220

Asp Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Ile His Asp
225 230 235 240

Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val
245 250 255

Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Leu Val Ser Ser Gly
260 265 270

Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Gln Gln Thr Gln Leu Phe
275 280 285

Thr Ser Gln Asp Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser
290 295 300

Asn Tyr Val Leu Ser Gly Phe Ser Gly Ala Ser Leu Phe Thr Thr Phe
305 310 315 320

Pro Asn Ile Gly Gly Leu Pro Gly Ser Thr Thr Thr Gln Ala Leu Leu
325 330 335

Ala Ala Arg Val Asn Tyr Ser Gly Gly Ile Thr Ser Gly Ser Ile Gly
340 345 350

Gly Ser Asn Phe Asn Gln Asn Phe Asn Cys Asn Thr Ile Ser Pro Pro
355 360 365

Leu Ser Thr Ser Phe Val Arg Ser Trp Leu Asp Ser Gly Ser Asp Arg
370 375 380

Gln Gly Val Thr Thr Val Thr Asn Trp Gln Thr Glu Ser Phe Glu Thr
385 390 395 400

Thr Ser Gly Leu Arg Cys Gly Ala Phe Thr Pro Arg Gly Asn Ser Asn
405 410 415

Tyr Tyr Pro Gly Tyr Phe Ile Arg Asn Ile Ser Gly Val Ser Leu Val
420 425 430

Leu Arg Asn Glu Asp Leu Lys Arg Pro Leu Tyr Tyr Asn Glu Lys Arg
435 440 445

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Asn Ile Glu Ser Pro Ser Gly Thr Pro Gly Gly Ala Arg Ala Tyr Met
    450                 455                 460

Val Ser Val His Asn Lys Lys Asn Asn Ile Tyr Ala Val His Glu Asn
465                 470                 475                 480

Gly Thr Met Ile His Leu Ala Pro Glu Asp Asn Thr Gly Phe Thr Ile
                485                 490                 495

Ser Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr Phe Ile
            500                 505                 510

Ser Glu Lys Phe Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu Gln Ser
        515                 520                 525

Asn Thr Thr Ala Arg Tyr Thr Leu Arg Gly Asn Gly Asn Ser Tyr Asn
    530                 535                 540

Leu Tyr Leu Arg Val Ser Ser Ile Gly Asn Ser Thr Ile Arg Val Thr
545                 550                 555                 560

Ile Asn Gly Arg Val Tyr Thr Ala Ser Asn Val Asn Thr Thr Thr Asn
                565                 570                 575

Asn Asp Gly Val Asn Asp Asn Gly Ala Arg Phe Ser Asp Ile Asn Ile
            580                 585                 590

Gly Asn Val Val Ala Ser Ser Asn Ser Asp Val Pro Leu Asp Ile Asn
        595                 600                 605

Val Thr Leu Asn Ser Gly Thr Gln Phe Asp Leu Met Asn Ile Met Leu
    610                 615                 620

Val Pro Thr Asn Ile Ser Pro Leu Tyr
625                 630
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Claims

1. A polynucleotide sequence encoding a lepidopteran-active toxin, wherein said toxin can be encoded by a polynucleotide sequence which comprises the polynucleotide sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 2, and the complementary sequences of SEQ ID NO. 1 and SEQ ID NO. 2.

2. A polynucleotide sequence which encodes a lepidopteran-active toxin from a *Bacillus thuringiensis* isolate selected from the group consisting of PS86I2 and PS192M4.

3. The polynucleotide sequence, according to claim 2, wherein said isolate is PS86I2.

4. The polynucleotide sequence, according to claim 2, wherein said isolate is PS192M4.

5. A polynucleotide sequence which encodes a lepidopteran-active toxin wherein said toxin comprises an amino acid sequence selected from the group consisting of SEQ ID NO. 4, SEQ ID NO. 10, and fragments thereof.

6. The polynucleotide sequence, according to claim 5, wherein said toxin comprises the amino acid sequence of SEQ ID NO. 4 or a fragment thereof.

7. The polynucleotide sequence, according to claim 5, wherein said toxin comprises the amino acid sequence of SEQ ID NO. 4.

8. The polynucleotide sequence, according to claim 5, wherein said toxin comprises the amino acid sequence of SEQ ID NO. 10 or a fragment thereof.

9. The polynucleotide sequence, according to claim 5, wherein said toxin comprises the amino acid sequence of SEQ ID NO. 10.

10. The polynucleotide sequence, according to claim 5, wherein said polynucleotide sequence comprises the nucleotide sequence of SEQ ID NO. 3 or a fragment thereof.

11. The polynucleotide sequence, according to claim 5, wherein said polynucleotide sequence comprises the nucleotide sequence of SEQ ID NO. 3.

12. The polynucleotide sequence, according to claim 5, wherein said polynucleotide sequence comprises the nucleotide sequence of SEQ ID NO. 9 or a fragment thereof.

13. The polynucleotide sequence, according to claim 5, wherein said polynucleotide sequence comprises the nucleotide sequence of SEQ ID NO. 9.

14. A polynucleotide sequence which encodes a lepidopteran-active toxin, wherein said toxin comprises an amino acid sequence selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 8, and fragments thereof.

15. The polynucleotide sequence, according to claim 14, wherein said toxin comprises the amino acid sequence of SEQ ID NO. 6 or a fragment thereof.

16. The polynucleotide sequence, according to claim 14, wherein said toxin comprises the amino acid sequence of SEQ ID NO. 8 or a fragment thereof.

17. The polynucleotide sequence according to claim 14, wherein said polynucleotide sequence comprises the nucleotide sequence of SEQ ID NO. 7 or a fragment thereof.

18. The polynucleotide sequence, according to claim 14, wherein said polynucleotide sequence comprises the nucleotide sequence of SEQ ID NO. 7 or a fragment thereof.

19. A lepidopteran-active toxin from a *Bacillus thuringiensis* isolate wherein said isolate is selected from the group consisting of PS86I2 and PS192M4.

20. The lepidopteran-active toxin, according to claim 19, wherein said isolate is PS86I2.

21. The lepidopteran-active toxin, according to claim 19, wherein said isolate is PS192M4.

22. A lepidopteran-active toxin wherein said toxin comprises an amino acid sequence selected from the group consisting of SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, and fragments thereof.

23. The lepidopteran-active toxin, according to claim 22, wherein said toxin comprises the amino acid sequence of SEQ ID NO. 4 or a fragment thereof.

24. The lepidopteran-active toxin, according to claim 22, wherein said toxin comprises the amino acid sequence of SEQ ID NO. 4.

25. The lepidopteran-active toxin, according to claim 22, wherein said toxin comprises the amino acid sequence of SEQ ID NO. 6 or a fragment thereof.

26. The lepidopteran-active toxin, according to claim 22, wherein said toxin comprises the amino acid sequence of SEQ ID NO. 6.

27. The lepidopteran-active toxin, according to claim 22, wherein said toxin comprises the amino acid sequence of SEQ ID NO. 8 or a fragment thereof.

28. The lepidopteran-active toxin, according to claim 22, wherein said toxin comprises the amino acid sequence of SEQ ID NO. 8.

29. The lepidopteran-active toxin, according to claim 22, wherein said toxin comprises the amino acid sequence of SEQ ID NO. 10 or a fragment thereof.

30. The lepidopteran-active toxin, according to claim 22, wherein said toxin comprises the amino acid sequence of SEQ ID NO. 10.

31. A transformed host which expresses a polynucleotide sequence encoding a lepidopteran-active toxin wherein said toxin comprises an amino acid sequence selected from the group consisting of SEQ ID NO. 4, SEQ ID NO. 10, SEQ ID NO. 6, SEQ ID NO. 8, and fragments thereof.

32. The host, according to claim 31, wherein said host is a plant or a plant cell.

33. An oligonucleotide primer selected from the group consisting of SEQ ID NO. 1 and SEQ ID NO. 2.

34. A method for controlling a lepidopteran pest wherein said method comprises contacting said pest with a toxin from a *Bacillus thuringiensis* isolate selected from the group consisting of PS86I2 and PS192M4.

35. The polynucleotide sequence according to claim 34, wherein said isolate is PS86I2.

36. The polynucleotide sequence, according to claim 34, wherein said isolate is PS192M4.

37. A method for controlling a lepidopteran pest wherein said method comprises contacting said pest with a toxin comprising an amino acid sequence selected from the group consisting of SEQ ID NO. 4, SEQ ID NO. 10, SEQ ID NO. 6, SEQ ID NO. 8, and fragments thereof.

38. The method according to claim 37, wherein said lepdiopteran pest is an *Ostrinia nubilalis*.

39. The method according to claim 37, wherein said lepdiopteran pest is a *Heliothis virescens*.

40. The method according to claim 37, wherein said lepdiopteran pest is a *Helicoverpa zea*.

INTERNATIONAL SEARCH REPORT

Internat l Application No
PCT/US 98/05081

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/32 C07K14/325 C12N5/10 A01N63/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 July 1998	07.08.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lejeune, R

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Internat' i Application No
PCT/US 98/05081

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DONOVAN W.P. ET AL.: "Amino acid sequence and entomocidal activity of the P2 crystal protein. An insect toxin from Bacillus thuringiensis var. kurstaki." JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 263, no. 1, 5 January 1988, MD US, pages 561-567, XP002071365 cited in the application see abstract see figure 2 -& DONOVAN W.P. ET AL.: "Addition and correction" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 264, no. 8, 15 March 1989, MD US, pages 4740-4740, XP002071366 see figure 4 96.1% identity in 1908 bp overlap with SEQ ID 3 85.6% identity in 1206 bp overlap with SEQ ID 5 89.6% identity in 1902 bp overlap with SEQ ID 7 89.5% identity in 1902 bp overlap with SEQ ID 9 ---	1,5,6,8, 10,12, 14-18, 22,23, 25,27, 29,31, 33,37,39
X	DATABASE EMBL Accession Nbr D86064, 28 June 1996 SASAKI J.: "Bacillus thuringiensis DNA" XP002071369 98.6% identity in 1908 bp overlap with SEQ ID 3 85.2% identity in 1206 bp overlap with SEQ ID 5 89.4% identity in 1908 bp overlap with SEQ ID 7 89.3% identity in 1908 bp overlap with SEQ ID 9 --- -/--	1,5,6,8, 10,12, 14-18, 22,23, 25,27, 29,31,37

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INTERNATIONAL SEARCH REPORT

Internat' Application No
PCT/US 98/05081

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WIDNER W.R. & WHITELEY H.R.: "Two highly related insecticidal crystal proteins of Bacillus thuringiensis subsp. kurstaki possess different host range specificities." JOURNAL OF BACTERIOLOGY, vol. 171, no. 2, 1989, pages 965-974, XP002071367 cited in the application see abstract see figure 2 88.3% identity in 1908 bp overlap with SEQ ID 3 85.8% identity in 1206 bp overlap with SEQ ID 5 92.7% identity in 1902 bp overlap with SEQ ID 7 92.7% identity in 1902 bp overlap with SEQ ID 9	1,5,6,8, 10,12, 14-18, 22,23, 25,27, 29,31,37

X	WU D. ET AL.: "Sequence of an operon containing a novel delta-endotoxin gene from Bacillus thuringiensis." FEMS MICOBIOLOGY LETTERS, vol. 65, no. 1, 1 June 1991, pages 31-35, XP002071368 cited in the application see abstract see figure 1 86.3% identity in 1006 bp overlap with SEQ ID 3 98.9% identity in 1873 bp overlap with SEQ ID 5 86.6 % idetity in 999 bp overlap with SEQ ID 7 86.4% identity in 999 bp overlap with SEQ ID 9	1,5,6,8, 10,12, 14-18, 22,23, 25,27, 29,31,37

X	WO 96 05314 A (MYCOGEN CORP) 22 February 1996 cited in the application see page 5; table 1	2,3,19, 20,34,35

X	WO 93 14641 A (MYCOGEN CORP) 5 August 1993 cited in the application see page 24, line 6 - line 8	2,4

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INTERNATIONAL SEARCH REPORT

Inte ional application No.
PCT/US 98/05081

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 3,8,9,12,13,20,29,30,35 (all complete) 1,2,5,19,22, 31,32,34,37-40 (all partial)

A lepidopteran-active toxin from the Bacillus thuringiensis isolate PS86I2, a polynucleotide sequence encoding such a toxin and the use of the toxin to control a lepidopteran pest.

2. Claims: 4,6,7,10,11,21,23,24,36 (all complete) 1,2,5,19,22, 31,32,34,37-40 (all partial)

A lepidopteran-active toxin from the Bacillus thuringiensis isolate PS192M4, a polynucleotide sequence encoding such a toxin and the use of the toxin to control a lepidopteran pest.

3. Claims: 15,25,26 (all complete) 1,14,22,31,32, 37-40 (all partial)

A lepidopteran-active toxin from the Bacillus thuringiensis isolate HD573, a polynucleotide sequence encoding such a toxin and the use of the toxin to control a lepidopteran pest.

4. Claims: 16-18,27,28 (all complete) 1,14,22,31,32, 37-40 (all partial)

A lepidopteran-active toxin from the Bacillus thuringiensis isolate HD525, a polynucleotide sequence encoding such a toxin and the use of the toxin to control a lepidopteran pest.

5. Claim : 33

An oligonucleotide primer selected from the group consisting of SEQ ID 1 and SEQ ID 2.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat' l Application No
PCT/US 98/05081

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9605314 A	22-02-1996	US 5686069 A	11-11-1997
		AU 3324795 A	07-03-1996
		CA 2196080 A	22-02-1996
		EP 0776368 A	04-06-1997
		JP 10504196 T	28-04-1998
WO 9314641 A	05-08-1993	US 5273746 A	28-12-1993
		AU 3427493 A	01-09-1993
		CA 2129107 A	05-08-1993
		EP 0626809 A	07-12-1994

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